EFFECTS OF MOLECULAR MASS OF A PRIMING RESIN ON BOND STRENGTH TO DENTIN

By

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by

Nicola Dean Richards

This dissertation is dedicated to my parents, Dean Boyd Richards, and the late Alice Francis Richards. Without them and without their lifelong input and examples, neither this dissertation nor I would have happened. The dissertation is also dedicated to Eleanor Needham Hempy Meggitt, whose life exemplified the grace and dignity towards which a human being should strive.

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This dissertation analyzed one of the factors that enhance the strength of the bond to dentin of polymeric composite restorative materials. It was hypothesized that increasing the molecular mass of the primer would increase the strength of the bond between tooth and composite restorative material. The objective of the research was to synthesize an oligomer with similar side groups, and vary the molecular mass of this oligomer. The first step in this project was the synthesis of a novel oligomer for use as a primer (initial polymeric component applied to the tooth to enhance bonding). The oligomer synthesized can be altered by increasing the molecular mass or by changing the number of side groups that are attached to the oligomer. The general reaction involves two steps. The first reaction is the copolymerization of maleic anhydride and tert-butyl methacrylate. The

second step is the addition of 2-hydroxyethyl methacrylate across the anhydride ring.

The effects of molecular mass of the primer were examined by changing the number of units that are polymerized before the primer is placed on the tooth. The relative (to polystyrene standards) molecular masses of 886, 998, 3366, 4556 and 5882 were tested in a standard dentin bonding test (shear). There was not a statistically significant difference ($p \leq 0.05$) between the two larger molecular masses and there was no statistically significant difference between the samples with mean molarcular mass of 886 and 3366 ($p \leq 0.05$). There was a statistically significant difference between the all other pairwise comparisons ($p<0.05$).

CHAPTER 1
INTRODUCTION

Since the development of modern resin composite restorative materials, a steady stream of new adhesive agents has been introduced to the market. Adhesives that attach to the microscopic irregularities of etched enamel were the first adhesives available. In the past two decades, dentin adhesives have begun to predominate. The rapid introduction of new products has meant that clinical results are often not available to substantiate or deny claims made by manufacturers when the products are introduced. Changes in formulations have been made to correct problems or difficulties from previous products. There have also been changes in the delivery systems. Today it is accepted that a primer must have a hydrophilic component in order to react well with the fluid-containing dentin and an organophilic component to interact with the organic polymers of the restorative materials. These primers are surfactants (contraction of "surface-active agent"), which are molecules in dilute concentrations that are able to adsorb onto a surface (dentin) and alter the free energy of the surface (Rosen, 1989). The primer allows the organophilic resin and composite to attach to the hydrophilic surface of the dentin.

It is desirable to advance beyond the search for new and different surfactants to learn which other features of a prospective bonding system make positive contributions to the adherence of a restoration to tooth structure. This dissertation will explore the effects that the molecular mass of the initial resin (the primer) have on the *in vitro* shear bond strength to dentin. Before the effects of one particular component can be evaluated, confounding factors should be reduced. Though it would be possible to compare three or four commercially available primers that have different molecular masses, the final bond strength might be more strongly affected by the larger number of hydrophilic side groups on one agent, or the lower viscosity of another. To minimize interference from extraneous factors, a copolymer was synthesized to allow discrete changes within the same family of molecules. The synthesized oligomer has the potential of being a good primer – that is to say, it fulfills the currently accepted ideal of having both hydrophilic and organophilic components. Once synthesized, this oligomer can be altered to explore the effects of a variety of changes on the bond strength to dentin.

During the past decade, microscopic has allowed visualization of the interface between the tooth and the polymers that are applied as restoratives work (Van Meerbeek, 1993), (Perdigão & Swift, 1994), (Dickens-Venz & Dickens, 1992). Polymers can be seen to penetrate between the exposed collagen fibers of the etched dentin and to penetrate down the tubules. Changes in molecular mass of the primer are likely to affect the primer diffusion and collagen/tubule infiltration.

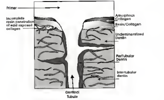


Figure 1 1 Penetration of etched dentin by primer

For some polymers the penetration is complete and there appears to be intimate contact with the native hard tissue For other polymers there remains an area of collagen nearest the unaltered dentin that does not contain polymerized resin (as on the left side of the tubule in Figure 1 1) or there is not a close adaptation of the polymer to the tubule walls In the case of an area of collagen that is not encased in polymer a channel exists for the infiltration of staining fluids and debris and for bacterial egress The ultimate sequela of such channels is the need to replace the restoration

A novel oligomer is proposed to examine factors that contribute to the success or failure of bonding resin composites to dentin The factor explored in this research project is the molecular mass of the initial resin (the primer) placed on the dentin. The hypothesis of this study is that there will be a change in the strength of the bond to dentin with changes in the molecular mass of the primer. The mechanical properties of polymers (such as strength and modulus) increase with increasing molecular mass. In contrast the logarithm of the diffusion coefficient is inversely proportional to the size of the molecule (Sperling, 1992). These two conflicting trends mean that increasing the molecular mass of an oligomer will only increase the performance of a dentin adhesive within the range that allows rapid diffusion (≤ 30 s) into the etched dentin. It is expected that there will be a maximum in the graph of molecular mass versus bond strength of a primer. As the molecular mass continues to increase, the slower diffusion into etched dentin and dentinal tubules indicates that bonding does not occur and so increases in mechanical properties of the materials are unimportant. Increases in molecular mass beyond this range will bring no change in bonding performance.

The specific aims of this study are as follows:

1. Synthesize a copolymer using maleic anhydride and tert-butyl methacrylate.
2. Produce a series of different molar mass samples of this copolymer.
3. Characterize the copolymer to confirm the structure.

4. Analyze the adhesive potential of the copolymers (different molar masses) as primers in a bond strength (shear mode) test to dentin

Statistical analysis will be performed to determine if varying the molar mass of a primer between 600 and 6000 amu generates different bond strength values to dentin. Kruskall-Wallis comparison on ranks will demonstrate if any statistically significant differences exist between the mean bond strength of the groups. If there are differences, this will be explored using the Student-Neuman-Keuls multiple pairwise comparison test to identify groups whose mean bond strength value differences are statistically significantly different. In addition, regression models of the data will be generated.

CHAPTER 2
BACKGROUND

Methacrylates have been used in dentistry for more than 50 years. They were introduced as a heat cured denture base material during the 1930s (Tylman, 1942) (Peyton & Mann, 1942). Soon after the introduction of the methacrylates German chemists developed an activator-initiator system that could be used to polymerize these materials without the use of heat (hence the name 'cold cured' methacrylates). These materials were developed in Germany during the Second World War, so the cold cured methacrylates did not appear on the American market until the end of the 1940s (Paffenbarger, Nelsen, & Sweeney, 1953) (Paffenbarger, Wolcott, & Smith, 1951) (Smith & Schoonover, 1953) (Christie, 1951). These methacrylates aroused great expectations that an aesthetic material had been found that would not dehydrate and dissolve as the silicate cements did.

One of the chemists who worked on the self curing resins was the Swiss chemist Hagger, working for the Amalgamated Dental Company in Zürich and London. He applied for a patent in Switzerland on July 21, 1949 - a patent that was granted November 15, 1951 (Swiss patent 278 946, British patent 687,299 1951). This patent resulted in the commercial product Sevriton Cavity Seal. Although this revolutionary product was the first commercial product with the ability to bond to tooth structure, Hagger rarely receives the recognition he deserves for inventing the first dental adhesive. The system was based on glycerophosphoric acid dimethacrylate, which could be polymerized by the action of sulfinic acid in a 5-to-30 minute period at 20°C. In 1951, Hagger described the auto-acceleration of polymerization of a resin system that included components of modern restorative resin composites: methacrylic esters with polymer re-enforcements, an acid component, and tertiary amine and peroxide initiators (Hagger, 1951). Hagger also developed a phosphate-based bonding system because he, along with others (Paffenbarger et al., 1953), (Paffenbarger et al., 1951), (Smith & Schoonover, 1953), (Christie, 1951), had realized that polymerization shrinkage was a key problem with these materials. By developing a material that could form a chemical bond with the tooth surface, Hagger believed it would be possible to overcome this shrinkage.

With the objective of evaluating pulpal response to resin systems, a clinical study was conducted at the Eastman Dental Hospital, London, in 1952. Caries-free teeth that were scheduled for orthodontic extraction were restored with some of the commercial resins available at that time (Kramer & McLean, 1952). One system, Sevriton (Amalgamated Dental Company), had the unique addition of an adhesive (Sevriton Cavity Seal). When examined histologically, the teeth that had been restored with Sevriton and Sevriton Cavity Seal responded to various histological stains in unexpected ways. As the authors had restored some teeth with Sevriton only, they were able to demonstrate that Sevriton Cavity Seal altered the response to stains. They believed that the initiator for the polymerization of the resin was a sulfinic acid (most likely para-toluene sulfinic acid) and was the probable cause of the staining abnormalities. Specifically, the dentin responded to basic dyes, such as haematoxylin, and acidic dyes, such as aniline blue, in ways that demonstrated an "exaggerated form of calcified dentin." Clinical techniques for using the Sevriton system were described in 1952 (Kramer & McLean 1952) and represented the first attempt to bond self-curing acrylic resins to dentin using glycerophosphoric acid as an etchant, which also provided some form of chemical bonding via phosphate groups.

As more researchers became aware of the polymerization shrinkage problem, more ideas to handle that problem were presented. The same year that Hagger patented his curing system, Knock and Glenn (1951) with L.D. Caulk Co. patented a system for use in dentures and in restorative dentistry. The system included promoters for the polymerization or copolymerization of methyl methacrylates, which were predominantly nitrogen compounds that included a ring structure. The list of compounds included many tertiary amines and, oddly enough, included caffeine. The patent also stated that the monomeric component could be improved by the addition of finely divided polymers or various inorganic fillers. These fillers could decrease the effects of polymerization shrinkage. Several new directions were explored during the 1950s. For example, DeTrey developed an epoxy resin. Because of limited use in dentistry, the Swiss chemical company, Ciba, was allowed to produce and market the epoxy resin, sold under the name Araldite. Bowen explored the use of this epoxy when he first tried to develop a resin filled with quartz particles (Bowen, 1956, 1958).

In 1955, Buonocore made one of the most significant steps forward for the "bonding" era by showing that etching with phosphoric acid significantly increased the adhesion of acrylic resin to tooth enamel. The adhesion that develops on etched enamel has allowed the development of various methacrylate resins for restorations, for sealants, and for bonding of orthodontic brackets, porcelain veneers and prostheses. In his paper, Buonocore hypothesized that the increase in adhesion was associated with a tremendous increase in surface area, or the exposure of the organic framework of enamel – ideas that still influence our methods of adhering to dental hard tissues. He recognized that the acid treatment increased the wettability of the tooth surface by the resin, but guessed that polar phosphate groups could precipitate on the tooth surface to allow "chemical union". Even decades later, researchers occasionally attempt to demonstrate covalent bonding to tooth structure but in general, mechanical retention in microscopic features provides the primary source of adhesion of polymeric composite restorative materials to tooth structure. By leaching out some of the mineral content of the cut surface of dentin, acid etching increases the microscopic features available for resin infiltration.

Bowen, in the late 1950s and early 1960s, developed the restorative material bis-GMA (2,2-bis[4(2-hydroxy-3-methacryloxypropoxy) phenyl]propane) (Bowen, 1956, Bowen, 1958, Bowen, 1962, Bowen, 1965b, Bowen, Menis, Setz, & Jennings, 1985; Bowen & Rodriguez, 1962). Bowen's innovation was not only the resin, bis-GMA, but also the incorporation of silica particles coated with silane coupling agents. The silica particles decreased the effects of polymerization shrinkage of the bulk restoration and decreased the thermal expansion coefficient of the material. The coupling agent helped promote retention of the particles within the resin.

Soon after Bowen had developed the first modern composite he turned his attention to developing a dentin adhesive. He explored the use of the adduct of N-phenylglycine and glycidyl methacrylate (Bowen, 1965a). Having noted that early bonding deteriorated upon exposure to water, he looked for a surfactant (described as a surface-active agent) that contained a hydrophilic portion and an organophilic portion that could bond to the restorative material. He expected that a single molecule would interact with the two very different surfaces and would serve as a bridge between the restorative material and the hard tissue. At the same time, in Japan, researchers at Kuraray developed a phosphate based methacrylate believing that the phosphate group would interact with the mineral component of the dentin (hydroxyapatite) and the methacrylate group with the resin based restorative material. Unfortunately, none of the dental adhesives that were developed at the end of the 1960s and the early 1970s bonded strongly to dentin. This first generation of dentin bonding agents had shear strength values of less than 10 MPa.

During the latter part of the seventies, Kuraray developed a dentin adhesive that was expected to adhere well to dentin. The clinical trial of that product was conducted at Tokyo University ((Fusayama, 1987; Fusayama, Nakamura, Kurosaki, & Iwaku, 1979). The outcome of this study became a milestone in the development of dentin adhesives. Fusayama's group demonstrated that by etching the dentin surface with phosphoric acid before they applied the dentin adhesive, they could improve the bonding and sealing ability substantially. In this study by Fusayama and coworkers, they also demonstrated that human dentin could be etched for short times without causing any pulp complications (Fusayama, 1987; Fusayama et al., 1979). At approximately the same time, Nordenvall and Brännström (Nordenvall & Brannstrom, 1980a, 1980b) also demonstrated the efficacy and safety of short etching periods of dentin to improve adhesion.

Fusayama explained his success by assuming that the resin, before it polymerized, infiltrated the tubules and formed resin tags. This explanation, though, was disputed by another Japanese investigator, Nakabayashi (Nakabayashi, 1992; Nakabayashi, Kojima, & Masuhara, 1982a; Nakabayashi, Nakamura, & Yasuda, 1991; Nakabayashi, Takeyama, Kojima, & Masuhara, 1982b; Nakabayashi & Watanabe, 1983), who demonstrated a layer of interpenetrating resin in the exposed collagen. By use of electron microscopy he showed that an acid-resistant layer remained on the dentin surface after the placement of the bonding agent and formed a "hybrid layer" consisting of a mixture of polymerized resin and collagen fibers. Today, the hybrid layer is considered so important to bonding to dentin that "hybridization" is sometimes used to describe the effectiveness of present-day dentin bonding systems. Nakabayashi worked primarialy with resin systems based on the initiator tributyl borane (TBB) (Nakabayashi, Hahata & Masuhara, 1977) and the resin 4-methacryloxyethyltrimellitate anhydride (4-META).

The results of the Japanese studies during the late 1970s boosted the interest in dentin bonding and the development of the second generation of dentin bonding agents. These products consisted of a methacrylate group and an active group, e.g., phosphates, that were separated by a spacing group (mainly a hydrocarbon chain) (Munksgaard, Hansen, & Asmussen, 1984). With the second generation of products it was possible to reach bond strength values approximately 10 MPa.

The two decades that followed can be described as the "decades of dentin adhesives." The next evolutionary step was the third generation of dentin bonding agents. There are two third generation bonding agents that deserve to be mentioned. One of these, developed by Bowen and Cobb, consisted of the adduct of pyromellitic anhydride and glycidyl methacrylate (Bowen & Cobb, 1983). This bonding agent was used with N-(*p*-toyl)glycine endglycidyl metahcrylate (NTG-GMA) as the primer. The other bonding agent was developed by Munksgaard and Asmussen and consisted of a conditioner and a glutaraldehyde-hydroxyethyl methacrylate (HEMA) mixture (Munksgaard & Asmussen, 1984) (Munksgaard & Asmussen, 1985). This third generation of dentin bonding agents is the first generation of bonding agents that performed adequately intraorally. One product that evolved as a third generation bonding agent was Scotchbond 2 (3M Dental Products Co.), which was the first ADA (American Dental Association) approved dentin adhesive. The primer of Scotchbond 2 consisted of a 10% maleic acid-HEMA mixture, on which a resin monomer was placed.

During the early 1990s, dentin etching became generally accepted. The driving force behind that acceptance was the realization that the key to dentin bonding was to demineralize the dentin surface and infiltrate it with a resin system. That awareness and Fusayama's claim from 1979 that dentin etching did not cause any pulp complications, provided strong arguments to convince the profession that it was acceptable to etch dentin. A major player in that process was Kanca, who was a strong promoter of dentin etching. A few of his papers in the early 1990s had a major impact on the practice of bonding to tooth structure. One paper dealt with luting a fractured incisal enamel-dentin corner to a tooth with a large pulp exposure (Kanca, 1993). He succeeded in attaching the incisal corner by etching the fractured tooth surface then using a dentin adhesive. After one year of observation time, he showed that the pulp had survived the treatment. The other papers of significance demonstrated that dentin bonding could be improved by bonding to moist rather than to dry dentin (Kanca, 1992b), (Kanca, 1992a). Thus during the middle part of this decade, dentin bonding finally became acceptable. The bonding system used at that time consisted of an etchant (often 30-35% phosphoric acid), a primer (an amphoteric monomer dissolved in a suitable solvent), and a bonding resin (often a mixture of the monomers used in the primer and the composite). These systems produced "bond strength" values above 20 MPa and were marketed as the fourth generation of bonding agents.

Unfortunately, dentists in general had a hard time learning to use these products efficiently, which resulted in the development of the so-called fifth generation of dentin bonding agents. These products, represented by products such as Single Bond®, consist of an acid (35% phosphoric acid) and a mixture of the primer and the bonding adhesive. By combining the primer and the bonding agent, some potential errors, e.g., use of the dentin adhesive before primer, could be eliminated. However, by having the primer mixed with the bonding adhesive, these materials often require a longer application time to allow the primer to leave the dentin adhesive phase and diffuse into the collagen web. Unfortunately, many clinicians seem to believe that these systems are easier and faster and, therefore, do not allow the resin to diffuse for a sufficient time. Because of this insufficient diffusion time, the outcomes for the fifth generation bonding agents are often not as good as they were for the fourth generation of bonding agents.

In addition to the above bonding agents, a sixth generation of bonding agents has recently been developed. These products are self-etching bonding agents. A product such as Prompt L-Pop® is a typical example of a sixth generation bonding agent. This product consists of a unique delivery system, and during usage the different components (including the acid) are mixed together. A limitation of this product is that it interferes with activator-initiator systems present in dual cured composites, inhibiting the self-curing polymerization process. By analyzing the available shear strength values it may be concluded that it is unlikely that this product will outperform the clinical success of the fourth generation of bonding agents.

Because of the time it takes to conduct a clinical study, there is a need for a reliable, direct in vitro test for resin bonding that is capable of predicting the clinical performance. Whether such a test exists can be disputed. Several papers have been written in attempts to correlate various material properties of bonded polymeric composite restorative materials to their behavior intraorally (Truong & Tyas, 1988) (Söderholm 1991), (Peutzfeldt & Asmussen 1992). Questions that need to be addressed concerning the long-term efficacy of these restorations were raised (Söderholm 1995). In this paper, Söderholm emphasized that variations in dentin substrate could play a significant role. For example, during most in vitro testing, the bond is located in a dentin region that has not been close to a carious lesion. Clinically, though, most restorations are bonded to either sclerotic dentin or caries-affected dentin. Shear, tensile and microtensile bond tests are used to approximate clinical performance. The shear strength test used by Hansen and Asmussen (1985) consisted of using the bonding agent to adhere a short composite cylinder to the surface of extracted teeth (human or bovine). A quantifiable force was then applied (via a universal testing machine) to the cylinder until it fractured away from the tooth As the shear strength exceeds 25 MPa failures often occurred via the fracture of a piece of dentin From a clinical point of view such failures are very rare One might question the clinical meaning of a "bond strength" exceeding 25 MPa if the failure occurs primarily in dentin.

The polymerization shrinkage of polymeric restorative materials is of ongoing concern This early contraction of the restoration can be expected to induce stresses in the bonding agent and thereby cause marginal gaps between restoration and tooth structure Hansen and Asmussen (1985) targeted these gaps in a microgap test that led to the evaluation of polymerization shrinkage against the bond to the surrounding dentin walls in a circular pit restoration They have used the same approach in other studies (Hansen & Asmussen 1989) If a large bulk of restorative material is placed in one increment and cured, stresses at the tooth interface may lead to debonding along the cavosurface margin for some portion of the margin The extent of this microgap and its maximum width may represent the ability of the resin/restorative material to resist or compensate for these polymerization shrinkage stresses

In bonding to dentin the smear layer which consists of hydroxyapatite fragments and burnished and torn collagen is removed The smear layer is removed during the etching procedure and the underlying dentin is partially demineralized exposing a collagen network and opening and widening of the orifices of the tubules (Nakabayashi & Takarada 1992) Both the collagen network and the tubules are then infiltrated by the primer/bonding resins Work by Gwinnett and coworkers has shown that infiltration of the dentin surface with and without tubule infiltration may retain a composite restoration even after removal of the exposed collagen (Gwinnett, 1994), (Gwinnett, Tay Pang & Wei, 1996). Regarding the resin infiltration process many researchers have shown how important it is to keep the surface moist. As mentioned earlier Kanca, and also Gwinnett (Gwinnett, 1992 Gwinnett & Kanca 1992) showed that bonding to moist dentin increased the bond strength The explanation given is that desiccation of the dentin surface results in a collapsed and densified collagen layer through which the resins cannot easily diffuse In studies conducted on dentin surfaces from which collagen had been removed Kanca and Gwinnett found the same results These surfaces, too, should remain moist before the bonding resin is placed A possible explanation is that desiccation results in the formation of a precipitate that seals some of the microporosities These precipitates may hinder the bonding resin infiltration of the rough surface or the resin may infiltrate the precipitates that are not bound to the underlying dentin

It is obvious that a key problem of bonding to dentin is how readily the resins can diffuse into the microstructure of the dentin surface The effect of this diffusion into collagen was quantitatively examined by Gwinnett (Gwinnett, 1993) Gwinnett attributed an increase of one third of the bond strength to the diffusion into the etched collagen over strength gained from penetration into the dentin tubules Penetration into dentin tubules has been demonstrated (Van Meerbeek, 1993) (Perdigao & Swift, 1994) (Titley, Chernecky, Chan, & Smith, 1995) (Titley, Chernecky, Maric & Smith, 1994) producing "resin strings" analogous to the resin tag formation seen in etched enamel.

The chemistry of the primer placed on etched dentin should have a profound effect on its diffusion into the etched microstructure. Early work by Pashley (Pashley & Livingston, 1978) found that the size (molecular mass) of a molecule affected its diffusion through dentin. A series of molecules (including albumin, urea, dextran) with radii varying from 1.97 Å to ~37 Å (hydrodynamic radius) was monitored for diffusion rates through dentin discs. The molecule examined fit the linear regression model in equation 1 with an r value of –0.94.

$$\log(permeability) = -0.584(\log M_w) - 2.62 \qquad (1)$$

In the series of molecules examined, fluoride and chlorhexidine data did not fit well to this line. The diffusion coefficients of fluoride and chlorohexidine were much lower than expected and the authors hypothesized that this implied binding to dentin. Thus, there is the suggestion that the chemical nature of a molecule may effect the diffusion coefficient with regard to dentin. The inclusion of hydrophilic groups increased the ability of a resin to penetrate the tubules and the intertubular collagen (Venz & Dickens, 1993). Gwinnett found varying responses to moist dentin among commercial bonding agents with differing chemistries (Gwinnett, 1992). These studies emphasize the importance not only of molecular mass, but also of the primer chemistry on diffusion into the dentin microstructure.

The substitution of glycerol dimethacrylate for methacrylate produced an increase in the bond strength (Dickens, Venz & Dickens, 1992). When a resin that has the functionality of dipentaerythritol penta-acrylate (PENTA-P) is examined, it seems possible that the methacrylate groups may actually sterically hinder each other so that not all of 10 functional entities can react with other chains. Furthermore, reaction of high functionality oligomers with each other may cause rapid gelation that prevents diffusion into the deepest portions of the collagen web or tubules by free radicals. Without free radicals, the primer will not polymerize and will leach out of the dentin, producing a channel for leakage between the restoration and the dentin.

A very viscous polymer such as Bis-GMA, or possibly dipentaerythritol penta-acrylate, is improved by the addition of smaller molecules that may act as diluents (Venz & Dickens, 1993), (Peutzfeldt & Asmussen, 1996). Asmussen's group evaluated the effect of diacetyl and propanal when added to resins (Asmussen & Uno, 1992; Asmussen & Uno, 1993). Increasing additions of either chemical increased resin conversion. Increasing the percent additive up to 16% improved the hardness, but beyond that level, it decreased the hardness. This implies an additional role of a plasticizer for these molecules (diacetyl and propanal). Peutzfeldt and Asmussen's results (1996) imply an optimum level for plasticizers/crosslinkers (or at least these plasticizers) in dental resin that may be in the range of 15%

In view of Pashley's work concerning the molecular size effect of molecules on diffusion (Pashley & Livingston 1978) and Asmussen's theories of solubility parameters of resins (Asmussen & Uno, 1993), (Asmussen, Hansen, & Peutzfeldt, 1991), the geometry and chemistry of a primer should effect its diffusion into the microstructural features of etched dentin Such an approach can be understood if the collagen web is viewed as a polymer covering the dentin surface with the key objective of dentin bonding being to allow a monomer to diffuse into that polymer and form an interpenetrating network It is well known from polymer chemistry that a liquid will soften and penetrate a solid nonporous polymer surface if the liquid has about the same polarity and solubility parameter as the polymer This would suggest that a given etchant conveys to the dentin surface specific polarity and solubility parameters and that the dentin adhesive should match these parameters in order to penetrate and form a hybrid layer In a study conducted by Asmussen et al (1993) the investigators found that the values of solubility parameter (δ) and fractional polarity (P) that gave the highest bond strength values were those that matched the δ and P values of the conditioned dentin

Small (1953) developed a method of estimating the solubility parameter of a polymer In order for a polymer to dissolve in a liquid, the cohesive energy density of the two must be similar "The molar cohesive energy is the energy necessary to break all intermolecular contacts in a mole^{-1}. The application of various thermodynamic equations for free energy and heat of mixing leads to the equation

$$\delta = (E/V)^{1/2} \qquad (2)$$

where E is the molar cohesive energy and V is the molar volume. The solubility parameter (δ) is an additive property, so summation of the contribution from small molecular units may be used to generate an approximation of the value for a whole molecule (or polymer) (Carraher, 1996).

$$\delta = \left(D\sum G\right)/M \qquad (3)$$

Equation 3 relates the solubility parameter (δ) to the density (D), monomeric weight (M) and Small's calculated molar attraction constants (G). By using published vapor-pressure and heat of vaporization data, Small calculated molar attraction constants for the cohesive energy density contribution of a small molecular unit (such as a methyl group) to the total cohesive energy density of a polymer (Small, 1953). Summing the values appropriate for a given molecule permits the approximation of the solubility parameter for that molecule.

The effects of various surface treatments on dentin and their ability to interact with the primer are obviously very important variables to consider when any dentin bonding agent is developed (Asmussen & Uno, 1993). Thus, molecular groups along the bonding agent can alter the ability of the resin to infiltrate the collagen web and wet the hard tooth surface beneath the collagen web. For example, hydroxyethyl methacrylate (HEMA) and the carboxylic side groups of polyacrylic acids (PAA) affect the wetting of the dentin and the subsequent wetting of the primed surface by the bonding agent and composite restorative material. Each of these factors affect the final bond strength.

In addition to wetting and diffusion ability, the resin must also cure efficiently within the collagen structure. By increasing the functionality of the resin, Dickens-Venz explored the effect of doubling the functionality of a primer, pyromellitic dimethacrylate (PMDM), by substitution of glycerol dimethacrylate for the methacrylate groups (Dickens-Venz & Dickens, 1992). This substitution produced an increase in the bond strength. It was noted that diluents increased the degree of cure of the pyromellitic glycerol dimethacrylate. Stanabury reported similar results with bis-phenol-A glycerol methacrylate (Bis-GMA) (Stansbury & Antonucci, 1992).

In addition to the resins used for bonding, solvents are also added to the bonding resin, primarily to decrease the viscosity of the bonding monomer. The solvent is often acetone or a water/ethanol mixture. Acetone as a solvent is particularly useful when excessive moisture is present on the dentin surface (Jacobsen & Söderholm, 1998). The benefit of acetone is that it acts as a water chaser. However, there are also drawbacks with the use of acetone as a solvent. First, it evaporates easily, which results in a change in the monomer: acetone ratio and thus a change in the viscosity of the bonding system over time. Second, a bonding agent containing acetone may not be able to infiltrate the collagen before the acetone has evaporated. Fast evaporation results in densified collagen, which in turn reduces the bond strength. If, for example, an acetone based bonding agent is rubbed on a moderately moist dentin surface, the bond strength becomes weaker than if no rubbing was performed (Jacobsen & Söderholm 1998). This is likely caused by accelerated water evaporation and collagen densification, which decreases the ability of the bonding resin to completely infiltrate the collagen web.

The water/alcohol based solvents seem to be less technique sensitive (Jacobsen & Söderholm 1998). These solvents have the ability to reswell a collagen web that might have been somewhat desiccated before primer application. With such a solvent, bond strength is improved by rubbing the surface. However, if the surface is heavily moisture contaminated, the acetone based bonding agent will produce a higher bond strength.

A topic that has been little investigated is how monomer systems dissolve in different solvents. This is an important topic, because there is a certain risk that a monomer system over time might separate out from the solvent. If a dentist is not aware of this risk, he/she may not regularly shake the bonding agent bottle in an attempt to mix the different components. Instead the dentist may run into the problem that when he/she coats the dentin surface with what is believed to be a bonding agent, but in fact it is only a solvent.

After the surface has been coated with the resin system, it is important to remove the moisture originally present in the dentin as well as the solvent present in the bonding resin system This is achieved by blowing air on the dentin for a few seconds The bonding system must be cured before the composite is added In most modern systems, curing is achieved using light activation Thus, chemicals such as camphorquinone and dimethylaminoethyl methacrylate are mixed with the bonding resin, and when the resin is exposed to light with a wavelength around 468 nm free radicals are generated that initiate the polymerization Today, limited information is available regarding the degree of cure that is achieved within the hybrid layer and one can suspect that the optimal curing conditions have still not been achieved

The previous review has dealt mainly with the ability of resins to penetrate the collagen web exposed after etching Knowledge of the structure of the dentin substrate may help understand bonding to dentin As with many biological tissues the molecular structure and morphology of dentin is highly influenced by genetic and environmental differences

Dentin consists roughly of 65% inorganic salts 20% organic materials and 15% water (Provenza & Seibel 1986) Most of the inorganic materials consist of hydroxyapatite but some calcium carbonate is also present in the hard tissue The organic material is mainly made up of collagen, but some other proteins and organic compounds are also present Some of the latter proteins are key components in the mineralization process

Dentin is made by the odontoblasts, cells that line the interior dentin surface (Provenza & Seibel 1986) The odontoblasts are highly specialized fibroblasts that originally were in contact with the epithelial cells that became the ameloblasts. The two dental hard tissues, dentin and enamel, are laid down along the interface of these two cell types, extending in opposite directions. As a consequence, dentin is deposited from the dentino-enamel junction towards the pulp and, as the tooth ages, the dentin thickness increases. The odontoblasts produce collagen that is released from the top of the cells, adjacent to the transition to the odontoblastic process. The collagen fibers form a collagen matrix that after some time starts to mineralize. Since the odontoblasts continuously move toward the pulp, there is always a layer of unmineralized dentin adjacent to the odontoblasts, a layer that is called predentin.

As the hydroxyapatite {$Ca_{10}(PO_4)_6(OH)_2$} is deposited, two types of dentin can be identified. One type forms a tube around the odontoblastic processes. That dentin is called peritubular dentin and consists of finer hydroxyapatite crystallites than those deposited in the dentin between the peritubular dentin, the so-called intertubular dentin. The inside of the peritubular dentin is covered with a thin collagen film called the lamina limitans. There are also branches from the odontoblastic processes that exit the peritubular dentin as lateral canals, or canniculae (Provenza & Seibel 1986). Some of these branched dentin canals can even extend between tubules. The latter is particularly common in the mantel dentin, a layer of dentin located just under the dentino-enamel junction. Side canals are also more common in root dentin than in crown dentin. The side canals in general, extend from the tubules in an occlusal direction (Mjor & Nordahl 1996)

If dentin undergoes a carious assault the bacteria involved cause two damaging processes to occur First, the hydroxyapatite is demineralized and second, the microorganisms degrade the exposed collagen From a dentin bonding perspective this is a very important consideration Some of the salts precipitate deeper into the dentin forming so called sclerotic dentin while collagen degradation weakens the strength of the collagen web Thus, when dentin bonding is perfomred on a caries affected surface, the bond can be compromised If insufficient tissue is removed bonding relies on a more or less degraded collagen web Also, if the sclerotic dentin surface is exposed the acid cannot etch the dentin If the mineral component is not removed by the etchant, the collagen web may not be as easily exposed as it is under normal conditions In addition mechanical retention formed by resin infiltrating tubules and branches will not exist Thus dentin bonding to older and sclerotic dentin, or to caries affected dentin is not as reliable as bonding to younger or caries-free dentin

Another factor that effects bond strength is the distance between tubules As the pulp is approached, the tubule density changes (Provenza & Seibel 1986) increasing from about 8 100 per mm^2 near the dentino-enamel junction to 57,600 per mm^2 near the pulp chamber (Mjor & Nordahl 1996) This change in tubule density means that the collagen web area decreases nearer the pulp and the area of hypermineralized peritubular dentin increases. Tubules vary in diameter from 1 to 3 µm. In addition, there are branches from tubules that cover a range of diameters from 1 µm down to 50 nm (Mjor & Nordahl, 1996). These branches anastomose with other branches and with other tubules, providing an interconnected system throughout the dentin. All these changes in geometry mean that dentin bond strength decreases in deeper dentin layers.

Based on the prior discussion, the key principle behind dentin bonding is micro-mechanical retention, retention that is achieved via collagen infiltration or hard tissue infiltration (tubules, branches and other porosities). The first bonding agents were developed using compounds that could form some kind of bond to the hydroxyapatite surface via ionic interaction. Probably a more interesting concept was that proposed by Munksgaard and Asmussen (Munksgaard & Asmussen, 1985) when they attemped to bond glutaraldehyde to collagen and then attach HEMA and the composite to that bond site. Whether they succeeded or not is still an open question.

The benefit of bonding to collagen is that collagen permeates the dentin. Thus, a true hard tissue interpenetrating network bond formation would occur, promising a bond as reliable as the strength of the collagen. To succeed in bonding to collagen, the structure of collagen needs to be examined in greater depth.

Collagen is not a unique molecule but a generic name for a family of related proteins. The collagen in dentin is primarily a Type I collagen (Provenza & Seibel, 1986), (Kraus, Jordan, & Abrams, 1969). This collagen consists mainly of three amino acids, glycine, proline and hydroxyproline. Type I collagen molecules form discrete fibers. These fibers consist of fibrils with a repetitive banding pattern, which can extend across adjacent fibrils within a fiber. Type I collagen fibers are capable of assuming a rod-like form, approximately 300 nm in length and interacting with adjacent collagen molecules. Type I collagen itself is virtually insoluble in tissue fluids, and the molecules within a fiber are chemically cross-linked to each other. Each collagen molecule consists of three distinct polypeptide chains, containing about 1056 amino acids, called alpha (α) chains. Each of these α-chains is twisted into a left-handed helix with about three amino acids per turn. The three helical α-chains are then coiled around each other into a right-handed super-triple helix to form a rigid structure similar to that of a rope. The sequence of the predominant part of these chains is characteristic and consists of repeating tripeptides of glycine-X-Y where X and Y represent amino acids other than glycine. The X is often proline and the Y residue is often hydroxyproline. In the α-chains of Type I collagen there are 338 Gly-X-Y triplets repeated in sequence with an additional 32 amino acids at each end, known as telopeptides.

Type I collagen is a heterotrimer. Two of the α-chains are identical with the third chain being unique. The molecular composition can therefore be written as $(\alpha 1(I))_2(\alpha 2(I))$. As mentioned previously, the three α-chains in each collagen molecule are wrapped round each other to form a long rigid triple-helical molecule 297 nm in length. The short telopeptide sequences at each end of the α-chains remain free. It is the three repeating Gly-X-Y triplets in each chain that allow the three α-chains to form the triple-helical structure (Marks, Marks & Smith 1996). Glycine is the smallest amino acid and its replacement by any larger amino acid residue inhibits triple-helical formation because there is inadequate space. The three chains can no longer be tightly wound around each other because they are not in the normal triple helix structure. This structure of collagen also depends critically on the presence of high amounts of proline and hydroxyproline in the α-chains. Both of these amino acids differ from the other α-amino acids of proteins in that they are imino acids with a rigid cyclical structure, which limits rotation of the polypeptide chain backbone. Their presence is essential for the stability of the triple helix.

Intact triple-helical procollagen molecules are secreted via the Golgi apparatus whereupon they leave the odontoblast cell. The conversion of procollagen to collagen is catalyzed by two specific proteases that cleave the nonhelical amino end carboxyl domains of the molecule. The collagen molecules produced by cleavage of procollagen then spontaneously assemble into fibrils and fibers. What determines fiber size and morphology in each connective tissue is uncertain, but there is evidence suggesting that these initial stages of collagen fiber formation may occur within intracellular bays formed by the surface of the secreting connective tissue cell, which controls the initial fiber orientation.

The packing of Type I collagen molecules within a fiber is very precise and generates the repeat periodicity of 67 nm observed in suitably stained electron micrographs. Each molecule of 300 nm length is displaced laterally from its neighbor by a distance (D) of 67 nm. Each collagen molecule can thus be regarded as composed of four 67 nm segments with an additional segment of 32 nm, leaving a gap of 35 nm before the next longitudinally aligned collagen molecule. Along the length of each molecule the arrangement of neutral, positively, or negatively charged amino acids form a pattern with a repeat of 67 nm. Therefore, when one collagen molecule is laterally displaced by 67 nm with respect to another, a strong interaction will occur between them, explaining the fact that collagen molecules can self assemble into fibrils. It is in the gap region that hydroxyapatite mineral is first deposited in calcifying bone and dentin.

The collagen fibrils that form first have little tensile strength. Strength is introduced in the final post-translation modification. The side chain amino groups of certain lysine and hydroxylysine residues within the collagen molecule are oxidized by a specific copper-containing lysine oxidase, which requires pyridoxal phosphate as a cofactor. This reaction yields an aldehyde residue on the collagen α-chains (Marks et al., 1996). All the ensuing chemical reactions of this aldehyde appear to be spontaneous and generated by the local environment at the cross-linking sites.

In dentin, the arrangement of collagen fibers is predominantly parallel to the dentino-enamel junction, or perpendicular to the tubules. The fibers tend to extend radially out from the tubules. Though both of these conditions predominate, there are many randomly oriented fibers.

In summary, dentin bonding agents have evolved from a belief that different chemicals can be bonded to either hydroxyapatite or collagen. The big breakthrough, though, was the acceptance of dentin etching and moist bonding. These breakthroughs can be understood by accepting the fact that acid etching enhances mechanical interlocking by increasing the area for resin penetration within the dentin and possibly by altering the chemistry of the surfaces. Moisture decreases the risk that the exposed collagen structures will collapse or that precipitates will seal microporosities. By accepting the above bonding mechanisms it seems reasonable to assume that a resin system with enhanced collagen wetting ability can quite efficiently infiltrate the exposed collagen structure and form excellent mechanical interlocking with the collagen network. Once the resin has infiltrated the porous micro-architecture produced by etching and maintained by the water, the monomeric resin must, in turn, be wet by and dissolve into the subsequent bonding resin and composite restorative material. The oligomer synthesized for this dissertation was planned to provide options to fulfill these conditions, with the added feature of being alterable to adjust for the influence associated with molecular molar while maintaining the chemistry of the hydrophilic and hydrophobic side groups.

CHAPTER 3
RESEARCH DESIGN AND METHODS

The research presented in this dissertation includes the synthesis of a dentin primer, its characterization and evaluation. Monomer characterization was conducted using nuclear magnetic resonance (NMR) and fourier transform infra-red spectroscopy (FTIR), while the molecular mass was determined by gel permeation chromatography (GPC). The bonding ability was measured using traditional shear testing, and diffusion and infiltration ability was studied using scanning electron microscopy (SEM).

Purification

Maleic anhydride

Maleic anhydride (Sigma Chemical Co., St. Louis, MO, lot #39H5409) was recrystallized from chloroform (Perrin & Armarego, 1988). To make a supersaturated solution, 36 g of maleic anhydride were added to 214 g of chloroform (Optima, Fisher Scientific Co., Fair Lawn, NJ; lot # 991379). The top of the beaker was sealed with Parafilm (American National Can, Neenah, WI) and the solution heated for 30 minutes to 55°C on a magnetic stirrer (Laboratory Stirrer/Hot Plate, Corning, Corning, NY). The beaker was removed to the benchtop and allowed to cool at room temperature for 30 min. Then the beaker was placed in a container filled with ice for another 30 min. A 15 cm cellulose fiber paper (Fisher Scientific Co., cat. #9-801E) was placed in a funnel and the precipitate and solution were poured into it. After the solvent had drained off, 50 mL of cold chloroform were poured over the precipitate, twice. The precipitate that remained in the filter paper was transferred to a petri dish and placed in a vacuum oven held at 500 mm Hg for 18 h. After drying, the maleic anhydride was stored in a desiccator.

Azo isobutyronitrile

The initiator, azo isobutyronitrile (AIBN) (Aldrich Chemical Co., Milwaukee, WI 53433, lot #01414B1), was recrystallized in a similar fashion (Perrin & Armarego, 1988). The AIBN, 49 g, was added to 200 g of chloroform, forming a supersaturated solution. This solution was heated, with stirring, for 20 min to a temperature of 25°C. The beaker was allowed to cool at room temperature for 30 min, and then placed in an ice filled container for another 30 min. The cooled solution was filtered, using a cellulose filter paper. The remaining precipitate was rinsed with two aliquots of 50 mL of cooled chloroform, then transferred to a petri dish and placed in a vacuum oven (500 mm Hg) for 18 h. After drying in the reduced pressure, the AIBN was stored in a desiccator.

Tert-butyl methacrylate

Tert-butyl methacrylate (t-BMA) (Polysciences, Inc., Warrington, PA, lot #500286) was distilled under reduced pressure (Perrin & Armarego, 1988). A 300 mL rounded bottom flask was charged with 50 mL of tert-butyl methacrylate (Figure 3.1). The flask was heated by a heating bath containing glycerin to 68+/-5°C. The flask was connected to a condensing column that had ice water (0-5°C) running through the jacket. The condensing column fed condensate into a three-neck flask that was nestled in an ice bath. The second neck of the flask was attached to a manometer (Houston Glass Fabricating, Houston, TX 77011) that had a valve to regulate the level of vacuum. The third neck of the flask was stoppered. The manometer assembly was connected to vacuum-type distillation trap (Pyrex, England, #7729-28). The distillation trap was kept in a vacuum flask (1000 mL, Pope Scientific, Menomonee Falls, WI) filled with liquid nitrogen. From the distillation flask, vacuum tubing led to the pump (HP=1/3, Sargent-Welch, Skokie, IL), which was used to reduce the pressure to 56 mm Hg. The boiling point of *t*-BMA at 57 mm Hg is 66°C.

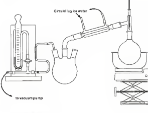


Figure 3.1 Distillation apparatus

Tetrahydrofuran

Tetrahydrofuran (THF) (Fisher, Scientific, Fair Lawn, NJ) was distilled from a flask containing sodium metal as a water scavenger. A 3000 mL round bottom flask that contained 75 mL of mineral oil with eight pieces of sodium metal (about 20 x 30 x 30 mm) was charged with 500 mL of THF and placed in a heating bath. The mineral oil was included in order to prevent moist air from reacting with the sodium. The flask was connected to a condensing column that had ice water (0-5°C) running through the jacket. The assembly was purged with nitrogen gas. A 500 mL round bottom flask was connected to the condensing column and nestled in an ice bath. Nitrogen gas was allowed to flow through the system. The tubing from the nitrogen tank had a bubbler tube attached to it that maintained the whole assembly at standard pressure, and prevented pressure build up within the flask. The THF was then raised to a temperature of 70+/- 5°C to distill it. The first ~25 mL were discarded and the last 50 mL were not used. The distilled THF was stored over molecular sieve (4 Å, 1.6 mm pellets, Aldrich Chemical Co., Milwaukee, WI, lot # 09316MG) in brown bottles. Prior to use, the molecular sieve was activated in an oven (Stabil-Therm, Blue M Electric Co., Blue Island, IL) at 200°C for 8 h. A nitrogen (gas) blanket was placed over the THF and the bottle capped.

Synthesis of the oligomer

First Step of the Polymerization

The backbone carbon chain was polymerized in a 500 mL round bottom flask A solution of t-butyl methacrylate in THF had maleic anhydride added to it on an equimolar basis To this the initiator, AIBN, was added in ratios to produce the varied molecular mass (see Table 3 1) Butanethiol (Aldrich Chemical Co , Inc , lot #00910BN) was added as a chain terminator The higher molecular mass oligomers were made using 3 mol% (compared to *tert*-butyl methacrylate content) AIBN and 0 3 mol% butanethiol while lower molecular mass oligomers had lower concentrations of the initiator but a higher ratio of terminator to initiator Within the various different concentrations of initiator and terminator, the reactants were dissolved in 200, then 300 and 400 mL of THF to further vary the molecular mass The reaction flask was purged with nitrogen gas and the polymerization carried out under nitrogen (a bubble tube on the nitrogen line meant that the reaction was carried out at standard pressure) The reaction was carried out in a heating bath (containing glycerin) at 75+/-5°C for 48 hours The solvent and excess monomer were then evaporated using a rotary evaporator (Rotavapor R Büchi-Laboratoriums-Technik AG Flawil/Schweiz) The concentrated solution of oligomer was rinsed with 50 mL of cold petroleum ether (Fisher Scientific Co   Fair Lawn, NJ) to precipitate out the oligomer After adding the petroleum ether the beaker was placed in an ice bath to enhance precipitation The precipitate was then redissolved in THF This rinse cycle of ether, then THF was repeated twice more. The final precipitate was filtered and dried under a vacuum of 50 mm Hg for 18 h.

Table 3.1 Ratio of Reactants for the Oligomers with Different Molecular Masses

Sample #	Mol % AIBN	Mol % butanethiol	mL solvent	M_w	PDI
342-072	1	2	200	943	1.02
342-063	3	4	200	954	1.03
342-067	3	4	150	998	1.04
362-091	3	0.3	300	3366	1.67
362-088	3	0.3	300	4556	1.49
362-094	2.3	0.35	400	5481	1.67
362-083	3	0.3	200	5862	1.65
362-042	1	4	200	882	1.02

The designation M_w stands for the weight average molecular mass and PDI is the polydispersity index, which is calculated by dividing the weight average molecular mass by the number average molecular mass (Sperling, 1992).

The polydispersity index is an indication of the distribution of the individual molecular masses within a polymer solution. From Table 3.1, it can be seen that the lower molecular mass oligomers in this series (#342-072, #342-067, #342-063 and #362-042) have very narrow distributions, while the higher molecular mass oligomers are more broadly distributed.

Reactivity Ratios

The reactivity ratio of two monomers can be utilized to predict the nature of a copolymer of the two (Carraher, 1996). A reactivity factor for one monomer that approaches zero implies the monomer will not polymerize with itself, while reactivity ratios equal to or greater than one imply that the monomer will readily homopolymerize. If the product of the reactivity ratios approaches zero, the two monomers are expected to form an alternating copolymer, while a reactivity ratio product that goes to one implies that the monomers will form a random copolymer. The reactivity of maleic anhydride in a methyl methacrylate system is 0.03 (Brandrup & Immergut, 1989) implying that it will not react with itself. In contrast, the reactivity of methyl methacrylate in a system with maleic anhydride is 3.5 (Brandrup & Immergut 1989). If the reactivity of *tert*-butyl methacrylate is similar to that of methyl methacrylate, then the *tert*-butyl methacrylate may be expected to polymerize with itself in blocks, while the maleic anhydride should only polymerize with the methacrylate. The copolymer of the two, therefore, is expected to include blocks of *tert*-butyl methacrylate with single units of maleic anhydride. It is likely that the two will react at a 4:1 ratio of *tert*-butyl methacrylate to maleic anhydride (i.e., n = 4 in Figure 3.2).

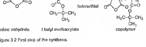
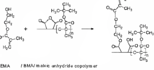


Figure 3.2 First step of the synthesis

Second Step of the Polymerization

Hydroxyethyl methacrylate (HEMA) was added in a molar concentration equal to the original amount of maleic anhydride The HEMA and copolymer were dissolved in THF and allowed to react for 18 h under a nitrogen environment The mixture was held at 45+/-5°C This oligomer with side groups was rinsed in petroleum ether and then THF three times and any final solvent removed under vacuum (56 mm Hg)



HEMA / BMA/ maleic anhydride copolymer

Figure 3 3 Second step of the polymerization

The copolymer was dissolved in acetone at 35% resin to acetone This solution was used as a primer in the dentin bonding tests

Characterization

Nuclear Magnetic Resonance

After each step of the synthesis the oligomer was dried For some samples this produced a very viscous liquid but for other samples a glassy solid was produced upon drying At least 200 mg of the oligomer were dissolved in 1 g of a deuterated solvent. Two solvents were used: dimethyl – d6 sulfoxide (DMSO) (Cambridge Isotope Laboratories, Andover, MA) or acetone –d6 (Cambridge Isotope Laboratories, Andover, MA). Both solvents were supplied in sealed 1 g ampoules with 1% v/v tetramethylsilane (TMS) added as an internal standard. The different solvents were used to examine for problems introduced by solvent/oligomer interactions. A Gemini 300 nuclear magnetic resonance with an Oxford 7T (300 MHz) magnet and Varian shims and Varian 5 mm probe was used.

The first step of the synthesis reacts maleic anhydride with tert-butyl methacrylate to produce the backbone of the final oligomer (Figure 3.2). In order to verify this reaction, peaks for the carbon-carbon double bonds should diminish in the spectra of the product. For proton nuclear magnetic resonance spectroscopy, unsaturated carbon-carbon bonds may have peaks at 1.5 to 2.5 ppm and at 4.5 to 6.5 (Skoog, Holler, & Nieman, 1998). ^{13}Carbon peaks from alkene carbon-carbon double bonds should occur between 100 and 150 ppm. Carbonyl peaks are seen between 160 and 175 ppm if included in an ester, while they will be nearer 180 ppm if they are part of an acid. The reaction signified above should have changes in the double bond peaks, but the carbonyl peaks should still be evident after the reaction.

The second step of the synthesis adds hydroxyethyl methacrylate (HEMA) across the maleic anhydride ring (Figure 3.3). The spectra of this oligomer should have changes in the carbonyl group peak and should add the double bond peak from the bond in HEMA. The hydrogen of the carboxyl group exchanges readily with the deuterium source so the peak is actually invisible in the solvents used Switching between the deuterated acetone and DMSO did not alter the absence of the peak for the carboxyl side chains

Reference spectra of the reactants were taken from Sadtler's carbon-13 NMR spectra (1976) and from Asahi Research Center's proton spectra (1985) These spectra were utilized to identify peaks attributed to different chemical groups within the structures To help identify the peak shifts associated with the reactants undergoing the first reaction, the ADEPT pulse sequence was used. This routine separates the spectrum into three spectra that represent the CH, the CH_2 and the CH_3 groups on separate graphs This allows more discrete identification of peaks – for the oligomer described here peaks from methyl groups overlay a septet of peaks from the deuterated solvent

Fourier Transform InfraRed Spectroscopy

The first step of the synthesis (Figure 3 2) should have Fourier transform infrared (FTIR) peaks that demonstrate the disappearance of double bond peaks Double bonds conjugated with a carbonyl will have a weak peak between 1650 cm^{-1} and 1600 cm^{-1}, and hydrogen attached to the alkene (as in methacrylate) will produce medium peaks in the 1850 cm^{-1} to 1800 cm^{-1} region (Bellamy, 1975), (Durig & Sullivan 1967) A disubstituted alkene can have a peak between 840 cm^{-1} and 790 cm^{-1} (the methacrylate) The five-member ring anhydride (maleic anhydride) has two carbonyl peaks at 1848 cm^{-1} and 1790 cm^{-1} (Bellamy, 1975) When comparing a spectrum of the reactants to the product of the first step, double bond peaks should decrease.

Alkenes would have a spectrum that might include the peaks shown in Figure 3.4.[1]

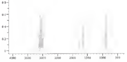


Figure 3.4 Some possible FTIR peaks for an alkene

The two peaks near 3000 cm^{-1} relate to stretching of the CH_2 bonds in an alkene. The peak between 895 and 650 cm^{-1} associated with out-of-plane deformation of the CH bonds, with the possible peak from 1600 to 1750 cm^{-1} occurring as an overtone of that vibration. The peak between

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[1] This spectrum and the following spectra were produced using IR Mentor Pro (BioRad Laboratories, Philadelphia, PA). They are representations of possible peaks.

1661 and 1639 cm^{-1} is caused by stretching of the carbon double bond The double bonds in the maleic anhydride will be shifted to slightly lower wavenumbers

The esters in the maleic anhydride and the methacrylate can give rise to the peaks in Figure 3 5

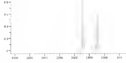

Figure 3 5 Some possible FTIR peaks for an ester

The peak at 1740 to 1715 cm^{-1} is due to stretching of an α,β unsaturated carbonyl group, while the 1210 to 1160 cm^{-1} is due to stretching of the C-O-C bond and may cause multiple bands

The ethers might generate the spectrum in Figure 3 6

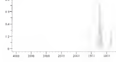

Figure 3.6 Some possible FTIR peaks for ethers.

The 1225 to 1200 cm^{-1} peak is due to symmetric stretching of the C-O-C bond, while the 890 to 820 cm^{-1} peak is due to asymmetric stretching.

The *tert*-butyl group would remain in all three spectra that are generated by the synthesis. The spectral peaks associated with this group are shown in Figure 3.7.

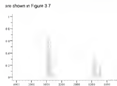

Figure 3.7 Some possible FTIR peaks for a *tert*-butyl group.

These peaks represent both symmetric and asymmetric stretching as well as deformation and backbone vibrations

As the reactants polymerize, it is expected that the peaks associated with the double bonds would diminish while the anhydride ring would remain intact, so the ester and ether peaks will remain, though they might shift.

The second step opens the anhydride ring and adds hydroxyethyl methacrylate (HEMA) (reaction shown in Figure 3 3) Double bonds reappear from the methacrylate moiety When the anhydride linkage is opened, an ester link is created with the HEMA molecule and a carboxyl side group is created on the backbone The maleic anhydride is a cyclic anhydride and will produce a peak in the region of 1780 cm^{-1} to 1760 cm^{-1} (Durig & Sullivan 1987) This peak should disappear but the ring is replaced by an ester linkage (to HEMA) and a carboxylic acid side group Thus, peaks for the ester will occur from 1750 to 1715 cm^{-1} and for the acid from 1450 to 1360 cm^{-1} and 1650 to 1540 cm^{-1}

The spectrum for this product will include the double bonds and the ester and ether linkage mentioned above, but will also include a carboxylic acid side group This reaction can generate the spectrum in Figure 3 8

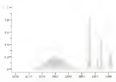

Figure 3.8 Some possible FTIR peaks for carboxyl moieties

The broad peak centered around 3000 cm^{-1} is caused by the stretching of the OH bond. Stretching of the carbonyl bond will give rise to the peak near 1700 cm^{-1}. The peaks around 1700 cm^{-1} and from 960 to 875 cm^{-1} are associated with out-of-plane deformation of the OH bond. The peak between 1320 and 1211 cm^{-1} is caused by stretching of the C-O-C bond.

The different sources used to evaluate the spectra give slight shifts to many of the groups, but in general agree on the approximate location of these peaks. The actual chemistry of neighboring atoms would be expected to cause shifts of the peaks compared to a generic spectrum.

Gel Permeation Chromatography

The gel permeation chromatography system used was produced by Waters (Milford, MA). It included a detector with a 410 differential refractometer and a 996 photodiode ray detector. The system had a 717 autosampler and a 600E system controller. The pump was a 610 model. A series of Phenomonex phenogel columns, all 300 X 4 6 mm, was used, starting with 5 X 10^5 Å, then 5 X 10^4 Å 500 Å, and 100 Å The samples were run for 40 minutes against a set of polystyrene standards

Solubility Parameter

The solubility of the oligomer was calculated using Small s molar attraction constants (Small 1953) These molar attraction constants are estimates of the cohesive energy density of small molecular units (such as a methyl or phenyl group) that may be summed over a large molecule (polymer) to estimate the molar cohesive energy of the molecule The square root of the ratio of the molar cohesive energy to the volume is the solubility parameter of the molecule The density of the oligomer was determined using a pycnometer (AccuPyc 1330 v1 Micromeritics, Norcross, GA) The density was measured to be 1 4709 g/cm^3 (+/- 0 0038)(three repeats of the measurement) By summing the contributions from each small unit of the proposed oligomer, the solubility parameter was calculated to be δ=11 66 This fits into a range that starts with hydrogen at δ=3 0 and goes through acetone δ=9 8, and methanol δ=14 5 to water at δ=23 4 (Carraher 1996) From these calculations, it is clear that the oligomer should not be soluble in water, and that acetone and methanol are the solvents of choice

Dentin Bonding Test

Teeth

Human teeth were sectioned to expose coronal dentin. The teeth had been stored in an aqueous solution of 0.05% thymol (Fisher Chemical Co., Fair Lawn, NJ, lot # 917385) for at least six months post extraction. The teeth were mechanically debrided to remove calculus and organic remnents. These teeth were mounted in dental die stone (Colorburst Die Stone, Econotek Inc., Orange, CA) and the dentin surface polished with silicon carbide abrasive papers to 1200 grit. In order to maintain the dentin surface in a plane perpendicular to the long axis of the sample, an aluminum slab with a hole just large enough to accommodate the sample was used to hold it perpendicular to the abrading surface.

Bonding

The polished dentin surface was acid etched for 15 s with 37% phosphoric acid gel (3M Dental Products, St. Paul, MN), then rinsed with distilled water for 20 s. The die stone surrounding the tooth was blotted with a single ply, lint-free tissue (Kimwipes EX-L, Kimberly-Clark, Roswell, GA 30076-2199). The actual exposed dentin was blotted lightly, once, to remove excess water. This left a visible film of water that was not thick enough to continuously cover the dentin surface. A 5 µL aliquot of primer was placed on the dentin and allowed to diffuse into the etched surface for 15 s. A second 5 µL aliquot was placed and allowed to diffuse in for 15 s. Any remaining solvent was blown off for 3 s. The air was supplied at 0.2 MPa and was blown in a stream perpendicular to the dentin surface from a syringe held one inch from the surface. A commercial bonding agent (Scotchbond MP Adhesive, 3M Dental Products, St. Paul, MN, lot OLP) was applied and a brush (Benda Brush, Centrix, CT) was used to gently spread the bonding agent. The brush was held almost parallel to the bonding surface, so that the sides of the bristles, rather than the tips, were glided across the resin surface. After 10 s, the bonding agent was light cured for 20 s. The curing light used (Spectrum 800, Dentsply, Milford, DE) had a built-in light intensity detector and it provided readings of 600 to 650 mW/cm^2 for each session that it was used. It was noted that any accumulation of bonding agent on the exit window of the light wand led to a drop in the intensity of the light. A stainless steel mold with a 3 mm diameter hole (2 mm in height or thickness of the stainless steel) was placed on the bonding agent and composite restorative material (3M Z100, 3M Dental Products, St. Paul, MN, lot #2001-0508) condensed in two increments, curing for 40 s after each increment (see Figure 3.9). The stainless steel mold has adhesive backed Teflon tape (#5490 HD 6241-09, 3M, St. Paul, MN) covering both sides to prevent any adhesion between the mold and tooth.

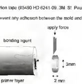

Figure 3 9 Mounted tooth and the stainless steel mold

This specimen was stored in a humid container for 1 d and tested to failure in a universal testing machine (Instron 5500R Canton MA using Merlin 4 31 software by Instron) at 0.5 mm/min. A stainless steel blade was attached to the crosshead The portion of the blade illustrated in Figure 3 13 takes the form of a hemi-cylinder with a bevel The flat portion of the rod was toward the tooth so that the point of the blade could be applied to the stainless steel mold close to the tooth The force was transmitted to the mold and through it to the upper circumference of the composite button The blade was placed as near the tooth as possible to prevent introducing a lever arm but care was taken to avoid wedging the blade between the mold and the tooth

In order to save teeth initial tests were done on human teeth used in previous bonding tests The dentin was reduced by 0 5 mm, then polished through 1200 grit with silicon carbide abrasive papers The removal of 0 5 mm was done to ensure that previously resin-infiltrated dentin was removed. These teeth were bonded with primers to determine the effects of various resin-to-solvent ratios For the final test teeth that had not been previously used for testing were mounted in die stone and then polished to 1200 grit silicon carbide abrasive paper Statistical analyses were performed on the results using Kruskall-Wallis test for multiple comparisons Statistical significance was set at $\alpha = 0\ 05$ Student-Newman-Keuls pairwise comparison was done to determine differences between the groups

Microscopy

Teeth

Human teeth that had been bonded and tested to failure were stored in a 0.05% thymol solution (2-isopropyl-5-methylphenol: Acros Organics, New Jersey; lot A010243101) and the teeth were sectioned to reveal the dentin bonding agent/restoration interface. The dentin-restoration interface was examined for tight adaptation of the copolymer to the dentin surfaces and penetration into the tubules and lateral branches and penetration into the full depth of the etched collagen. Micrographs of representative areas were taken for each treatment group. The drying necessary for scanning electron microscopy may introduce artifacts in the dentin-restoration interface, so the teeth were fixed in their hydrated state.

Teeth for microscopy were selected from each treatment group of the bonding test. In order to identify the bonded surface in cross-sections, a trapezoidal sample was cut from the area of bonding. The broad base of the trapezoid was the surface that had the primer and bonding agent on it (see Figure 3.10). When cross-sections were cut from this trapezoid, the bonded surface could easily be identified macroscopically and microscopically.

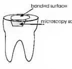

Figure 3.10 Sectioning of tooth for SEM microscopy.

Fixative Procedure for Teeth

The samples were preserved in a cacodylate solution. Initially a double strength buffer was prepared containing 0.2 M sodium cacodylic acid, sodium salt (Fisher Biotech, Fairlawn, NJ lot # 974823) and 8% sucrose (saccharose, Fisher Chemicals, Fair Lawn, NJ, lot # 975315) in distilled water. An aqueous solution of 8% para foramaldehyde (Fisher Scientific, Fair Lawn, NJ, lot #974603) was prepared by adding 1 to 3 drops of sodium hydroxide and heating to 70° C. The para-formaldehyde solution was adjusted to pH 7.3 to pH 7.4 by adding hydrochloric acid. A second double strength fixing solution was prepared with 15 mL of distilled water, 10 mL of 25% glutaraldehyde and 25 mL of the 8% para formaldehyde. The two double strength solutions were mixed 50:50 to produce the fixing solution. Samples were placed into the solution for 3 d. The cacodylate solution may only be stored for 2 weeks in the refrigerator.

After 3 d, the fixing solution was replaced with a single strength of the cacodylate/sucrose buffer. Samples were left in the buffer for 10 min. The buffer was replaced with fresh buffer and left for 10 min. This cycle was repeated for a third time. These fixed teeth were mounted in resin (PMMA) with the base of the trapezoid in the interior of the mounting ring (see Figure 3.11). This placement allows polishing of the cross-section, while protecting the bonded surface.

Figure 3.11 Placement of dentin sample in mounting resin

The sample was sectioned across the long axis of the dentin piece. This produced a cross-section of the bonded interface that included (centrally) tubules perpendicular to the bonded surface. The surface was polished, using wet SiC paper from 2400 to 4000 grit. This was followed by polishing with 0.025 µm diamond paste. After the final polish, the samples were cleaned ultrasonically (Henry Schein Inc., USA) for 9 min in distilled water. The water was changed and the sample recleaned (twice).

Etching Procedure

The polished surface of the sample still has a smear layer on it. Two methods of removing this layer were used. One half of each sample was etched with phosphoric acid (10% mass fraction) for 5 s, followed by immersion in sodium hypochlorite (5% mass fraction) for 5 min. The sample was then rinsed with water for 5 min. Using an argon gas, the dentin surface of the other half of each sample was etched for 5 min in the sputter coater (Desk II, Denton Vacuum, Moorestown, NJ) under a vacuum of 50 mTorr at 5 mA. After either etching procedure, the sample was sputtered with gold/palladium under a vacuum of 125 mTorr at 45 mA for 30 s. The samples were viewed in a scanning electron microscope (6400 SEM, JEOL, Peabody, MA) at an accelerating voltage of 5.0 kV. Images were recorded in digital files, using Digital Scan Generator 1 Plus (JEOL, USA).

CHAPTER 4
RESULTS AND DISSCUSION

The necessity of having an amphoteric molecule as a primary component for adhering the organophilic restorative material to the water filled dentin has been alluded to throughout this dissertation. The molecule described herein includes tert-butyl esters along the backbone as well as the double bonds on the hydroxyethyl methacrylate side group that should interact with bonding agents and composite restorative materials. Also included in the chain are carboxylates that provide a measure of hydrophilic groups to wet the dentin and to dissolve in the intrapulpal fluids that cannot be reliably excluded from the dentinal tubules. The chemistry of the oligomer would seem to make it an ideal candidate for a surfactant between tooth and restorative material.

The problem of penetrating the etched dentin may be viewed as a diffusion problem. Issues that predict the outcome of diffusion would be the diffusion coefficient and the time available for diffusion. For practical considerations in dentistry, the times involved need to be on the order of seconds or tens of seconds ($\leq$30 s). Factors that will impact the diffusion of the primer into the dentin include the mineralization of the dentin being bonded, the density of the tubules and their diameter, the area of the peritubular dentin, the presence of the odontoblastic processes in the tubules, and the condition of the collagen, to name a few. These tooth related factors will vary over the same tooth, from tooth to tooth and from patient to patient. Furthermore, the dentist may not pick the type of tooth he or she will restore – the only choice available to him or her is what material is used to restore the tooth presented for repair. Other factors that might impact the diffusion coefficient include the viscosity, chemistry and geometry of the diffusing species, i.e., the primer.

It is common in dentin bonding studies to use caries-free molars (preferably third molars) as substrates for bonding. It is expected that the variance of the study will be minimized by this focused selection of bonding substrate. Of all the teeth in the mouth, caries free third molars are the one tooth type that would not be reasonably restored with polymeric composites. Polymeric composites might be used to alter the shape of caries-free anterior teeth for esthetic reasons, but there seems no similar justification for the use of these materials on caries-free third molars. In taking this stand, the current dissertation may have a higher variance of the data than is commonly reported for dentin bonding studies. The teeth used for the tests included caries affected teeth, though bonding was never done to surfaces that were visibly stained due to caries. This tooth selection aligns with the universe of teeth that a dentist might need to restore with polymeric composites.

Synthesis

Nuclear Magnetic Resonance

The spectra of the two monomeric components were compared to reference atlases of spectra: Sadtler's carbon-13 NMR spectra (1976) and Asahi Research Center's proton spectra (1985).

The spectra presented in this section were made using deuterated acetone which contained 1%v/v of tetramethyl silane (TMS) as an internal reference. In proton NMR acetone has a peak at 2.05. In ^{13}carbon NMR acetone produces peaks in two regions: one single peak at 206.7 ppm and a septet at 29.9 ppm (Derome 1987). On occasion these peaks may overlap peaks of interest.

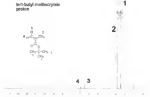

Figure 4.1 Proton NMR spectrum of *tert*-butyl methacrylate

The proton spectrum of one of the monomers used *tert*-butyl methacrylate (tBMA) is shown in Figure 4 1. There are two peaks at 6 and 5 5 that are generated by the hydrogens (cis and trans) attached to the carbon of the double bond At 1 86 is another peak associated with the double bond – this peak results from the hydrogens on the methyl group The peak at 1 472 represents the hydrogens of the *tert*-butyl group The peak at zero is the reference peak from TMS

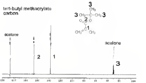

Figure 4.2 Carbon NMR of *tert*-butyl methacrylate

The two carbon peaks from acetone are well isolated in Figure 4.2 There is a peak at 206 7 ppm and the septet at 29 8 ppm is clearly a symmetrical set of peaks The carbonyl of the ester linkage is evident at 166 ppm There is a peak associated with the double bonded carbon at 138 ppm

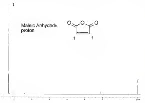

Figure 4 3 Proton NMR of maleic anhydride

Maleic anhydride, the other monomer is represented in Figure 4 3 This proton spectrum has a peak near 7 5 ppm that was caused by the hydrogens attached to the double bonded carbon

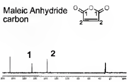

Figure 4 4 Carbon NMR of maleic anhydride.

A ^{13}carbon spectrum of maleic anhydride (Figure 4 4) appears very similar to the spectrum for *tert*-butyl methacrylate This spectrum has both a peak from the carbonyl of the ester linkage (~ 165 ppm) and the double bonded carbon (~138 ppm)

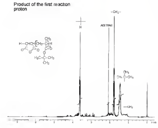

Figure 4 5 Proton NMR of the product of the first reaction of the synthesis

Notice in Figure 4 5 that the peaks around 7 to 6 ppm are missing (compared to Figure 4 1 and 4 3), the peaks from the single hydrogens are now on saturated carbons and have shifted near 3 6 ppm The peaks associated with the methyl groups (~ 1 1 and 1 5 ppm) remain The single methyl group is now attached to a saturated carbon and its peak has shifted to ~1 1 ppm

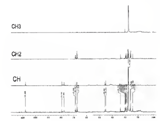

Figure 4 6 Carbon NMR of the product of the first reaction using the ADEPT routine

Figure 4 6 was made using a method that sorts the carbons according to the number of hydrogens attached to each In the bottom line the total spectrum for the solution is given The top line represents methyl groups, with two peaks near 30 ppm. On the second line are two multiple peaks near 70 ppm plus five peaks in the general region of 30 ppm, three of them being multiple peaks The third from top line represents carbons with a single hydrogen attached

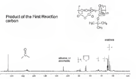

Figure 4.7 Carbon NMR of the product of the first step of the reaction

The ^{13}carbon spectrum of the first oligomer (Figure 4.7) is interesting for its lack of intense peaks between 180 and 160 ppm. The carbonyl and double bonded carbons of the two monomers all produce peaks in this region. The lack of intense peaks demonstrates the removal of the unreacted monomers from the solution after synthesis. Peaks in the region of 70 to 80 ppm are often caused by carbons involved in ether linkages. These reflect the ether linkages in the oligomer. The two double peaks around 70 ppm represent the solvent for the reaction (tetrahydrofuran or THF). There are more peaks in the region of 30 pm than might be attributed to acetone. Some of these peaks are from the methyl groups, both the single methyl and the tertiary methyl groups.

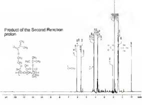

Figure 4.8 Proton NMR of the final product of the synthesis

Note the appearance of the two peaks near 6 and 5.5 ppm on Figure 4.8, when compared to the spectrum in Figure 4.5. These peaks are caused by hydrogens associated with double bonded carbons. These verify that the HEMA was added into the chain, providing double bonds that may react with the bonding agent and polymeric composite *in vivo*. Near 4 ppm are two peaks generated by hydrogens on carbons that are involved in ester linkages. The peak ~1.9 ppm is generated by the hydrogens attached to the carbon that was originally part of the double bond of the methacrylate. The peaks associated with the methyl groups remain

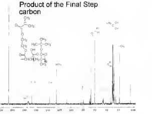

Figure 4 9 Carbon NMR of the final product.

In the spectrum of the final product (Figure 4 9), three peaks are evident between 170 and 120 ppm, representing the carbonyl (170 pm) and the double bonded carbons (135 and 125 ppm) Compare this spectrum to Figure 4 7, which lacks the peaks for the carbonyl and double bonds This spectrum (Figure 4 9) identifies a molecule with double bonds (for *in vivo* copolymerization with the bonding agent and polymeric composite restorative material) and carboxylate groups to enhance the hydrophilicity that will allow the dissolution into the hydrated dentin The spectrum is that of an amphoteric molecule

Fourier transform infrared spectroscopy

For the reactions that are described in this dissertation, the accomplishment of a particular reaction may be implied from the spectra taken before and after the reaction. The general location of peaks in the spectrum allows the identification of the molecular species that causes the peak. This may be predicted from the Equation 1.

$$v = (1/2\pi c)\sqrt{k/\mu} = 5.3\times10^{-12}\sqrt{k/\mu}$$

(1)

In equation 1, v is the vibrational frequency of the oscillation of the atoms of a molecule, *c* is the speed of light, *k* is the force constant for the bond between the atoms of the molecule, and μ is the reduced mass (Equation 2) of the atoms (Skoog et al. 1998).

$$\mu = m_1 m_2 / (m_1 + m_2)$$

(2)

By accepting the force constant, on average, to be 5 * 10² N/m for a single bond and 1.0 * 10³ N/m for a double bond, it can be predicted that

carbonyl molecules will have a vibrational frequency of 1600 cm-1

carbon-carbon double bonds will have a frequency of 1700 cm-1

carbon-oxygen single bonds will have a frequency of 1100 cm-1

As can be seen, two of the peaks that are of interest may overlap. The substituents on a molecule will shift the peak, as will interactions with nearby molecules that may generate weak intermolecular forces such as hydrogen bonding

The fourier transform infrared (FTIR) spectrograph contains the spectra of the original reactants the product of the first step and the product of the second step stacked from bottom to top (Figure 4 10)

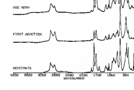

Figure 4 10 Spectra of the reactants and synthesis products

Note the small peak at about 1700 cm^{-1} and the height of the peak between 1760 and 1740 cm^{-1} in the spectrum of the reactants (bottom line) in Figure 4 10 These peaks reflect double bonded carbons There is a multiple peak between 1320 and 1200 cm^{-1} that changes from the bottom to the middle to the top spectrum The two peaks between 1760 and 1700 cm^{-1} not only change in intensity from the bottom to the middle and then to the top spectrum but the relationship of their heights changes In the top spectrum there is a subtle shift of the peak between 1720 and 1690 cm^{-1} The set of peaks around 3000 to 2800 cm^{-1} remains moderately constant in all three spectra but in the top spectrum (Add HEMA) there is a slight broad raising of the baseline The addition of a broad peak here reflects the addition of a carbonyl group to the oligomer

Scanning Electron Microscopy

The following micrographs are representative of the different molar masses of priming oligomer The samples were chosen from a high bonding molar mass group and a low bonding molar mass group Within in either group the highest bond strength sample was used to represent the group

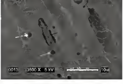

Figure 4 11 Micrograph of a tooth bonded to an oligomer with M_w 4556, chemical etch The black arrow points to the "resin string" that has penetrated the etched dentin tubule The white arrows point out the orifices of the lateral canals

In this micrograph (Figure 4 11), the sample has been etched with phosphoric acid followed by sodium hypochlorite The etching procedure first removes some mineral (in this micrograph, the peritubular dentin has been removed) and then removes the exposed collagen fibers In this micrograph the black arrow point to the resin strings in the tubules As can be seen at the top of the micrograph, there is no continuous layer of resin above the unetched dentin. This implies that the resin did not penetrate into the collagen web to produce an interpenetrating network of collagen/resin (generally termed the hybrid layer) If the resin had coated the collagen, the collagen would not have been digested by the sodium hypochlorite The surface of the resin strings does not reproduce the internal architecture of the dentin The diameter of the strings has diminished within the first 10 µm of penetration.

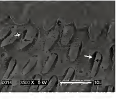

Figure 4 12 Micrograph of a sample bonded using an oligomer of M_w 998 Black arrows indicate resin strings penetrating the lateral canals, while white arrows point to the orifices of the lateral canals

Figure 4 12 is the micrograph of one of the highest bond strength samples (M_w of 998) In the micrograph the resin strings extending into the tubules have side extensions (identified by the black arrows) going into the lateral canals The etching procedure removes the mineral of the peritubular dentin Be aware that the lateral canals will extend from the tubules in an occlusal direction (which is toward the bonded surface), so that the side extensions are apical (away from the bonded surface) (Mjor & Nordahl, 1996) to the openings This means that the resin branch to the lateral canal does not closely match the lateral canal opening when the intervening peritubular dentin is etched away The layer of resin on top of the dentin surface can be seen to be continuous and to extend to the unetched dentin There is no exposed channel between the bonding resin and the intact dentin This implies that resin enveloped the collagen fibers, protecting them from the etchants

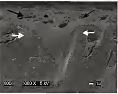

Figure 4 13 Micrograph of a sample bonded with an oligomer of M_w 5862 The sample was ion etched before microscopy The top area (above the white arrows) is the previously bonded surface, while the rest of the area is a cross section cut perpendicular to that surface The black arrows highlight tubule orifices

This micrograph (Figure 4 13) is from a sample in the low bond strength group (M_w 5862). This surface of the cross-section was ion etched. The etching procedure does not differentially etch the resin and the dentin as does the chemical etching procedure The area of the micrograph above the white arrows shows the previously bonded surface while the bottom portion is a cross section cut perpendicularly to the bonded surface In the previously bonded surface the orifices of the tubules (black arrows) appear not to have any resin and the intertubular dentin appears not to be coated In the cross-section portion, the cured resin appears in the tubules but is fractured below the surface of the dentin. From this micrograph it may be assumed that the resin did not reliably penetrate the etched intertubular dentin Some retention was gained from resin that penetrated the tubules.

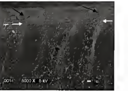

Figure 4 14 Micrograph of a bonded sample of an oligomer with M_w 998 The bonded surface is above the white arrows and the black arrows indicate the tubule orifices

In Figure 4.14, the sample was one of the highest bond strength groups (M_w of 998) Comparing this to micrograph 4 13, the bonded surface of the resin can be seen to be continuous with the resin obscuring the tubule orifices (black arrows) There is no discernible line between the resin and the unetched dentin or tubules It appears that the oligomer readily penetrates and then polymerizes in both the intertubular dentin and tubules This sample and the sample in Figure 4 12 were ion etched before microscopy

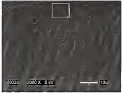

Figure 4 15 Micrograph of a sample bonded with an oligomer of M_w 998 Box outlines the area magnified in the next figures

Figure 4 15 pictures one of the high bond strength samples In the succeeding figures, the area in the box will be magnified

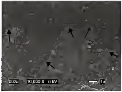

Figure 4 16 Higher magnification of Figure 4 15 The black arrows highlight the interface between the resin (top) and dentin

In Figure 4 16 the black arrows outline the interface between dentin and resin (top of micrograph) At this magnification (10,000 X), there is an intimate contact between the two Along some portions of the interface, the boundary cannot be distinguished

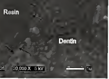

Figure 4 17 Higher magnification of Figure 4 16 Black arrows point out the intersection of the resin and dentin This area depicts the wall of a tubule

Figure 4 17 illustrates the close adaptation that characterizes the interface of the lower range of the molar mass oligomers examined The tubules will be between 1 and 3 μm in diameter (Mjor & Nordahl 1996) The layer of resin infiltration on this sample appeared to be ~ 3 μm thick (Figure 4 15)

Gel Permeation Chromatography

For the different combinations of reactants described in Chapter 3, the gel permeation chromatography (GPC) results are given in table 3 1 The designation M_w stands for the weight average molecular mass and PDI is the polydispersity index, which is calculated by dividing the weight average molecular mass by the number average molecular mass (Sperling 1992) The polydispersity index is an indication of the distribution of the molecular mass of the polymer solution From table 3 1, it can be seen that the lower molecular mass oligomers in this series (#342-072, #342-067 and #342-063) have very narrow distributions, while the higher molecular mass oligomers are more broadly distributed

Bond Strength

The bond strength to dentin was examined for five different molecular masses 882 998 3366 4556 and 5962 The graph of the raw data is pictured in Figure 4.13

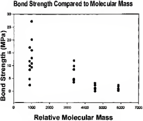

Figure 4 13 Relative molecular mass plotted against bond strength to dentin

The two lower molecular masses (682 and 998) appear to overlap in the graph The average bond strength values are given in Table 4 1

Table 4 1 Mean Shear Bond Strengths of Experimental Primers

Molar mass	n	Bond Strength (MPa)	Standard Deviation
682	6	9 06	5 197

998	6	16 07	6 711
3366	8	7 14	3 422
4556	6	1 23	1 301
5862	6	1 20	0 791

A Kruskall-Wallis comparison on ranks was done and there was a greater difference between the groups than would be expected by chance ($p<0$ 001) This test was followed by an all pairwise multiple comparison (Student-Newman-Keuls method) to identify statistically significant differences in means ($p<0$ 05) The only two groups for which the differences in means were not statistically significant ($p<0$ 05) were the 882 and 3366 molecular mass groups and the 5862 and the 4558 molecular mass groups In pairwise comparison the differences in means for the other groups were statistically significantly different ($p<0$ 05) A polynomial regression analysis was performed, producing the model in equation 4 1

Bond strength = -1 481 + (0 022 * Mw) - (0 0000084 * Mw^ 2) + (0 00000000061 * Mw^ 3) (4 1)

This model has a multiple coefficient of determination, R^2 of 0 608 A linear regression was also done generating the model in equation 4 2

Bond strength = 14 793 - (0 0025 * Mw) (4 2)

This model has a coefficent of determination, R^2 of 0 534

If a primer is to succeed, it must diffuse into etched dentin and must then be polymerized As an oligomer's molar mass increases its viscosity will increase (Sperling, 1992) according to equation 4 3

$$[\eta]=kM^{a} \qquad 4\ 3$$

Equation 4 3 (Mark Houwink equation) describes the relationship between intrinsic viscosity $[\eta]$ and molecular mass (M) The constants k and a are specific for a polymer/solvent combination and must be determined experimentally As the viscosity increases the diffusion rate will decrease In contrast, mechanical properties of a polymer will increase with increasing molar mass If both of these factors are working on the primer, it may be expected that some increase in molar mass will bring an increase in the strength (mechanical property) of the final composite while further increases effect a decrease due to exponentially increasing viscosity decreasing the diffusion rate It was hypothesized that some intermediate range of molar mass would provide the greatest bond strength of a priming oligomer The microscopy shown in this dissertation indicated that the higher molar mass oligomer did not penetrate and polymerize in the collagen web created by acid etching the cut dentin. The bond strength tests provided information that in the range of 800 to 3500 relative molar mass, this series of oligomers reached a maximum bond strength

Conclusions

I conclude that the molar mass of a priming oligomer is inversely related to the mean bond strength of a polymeric composite to etched dentin

The data reported here suggest that the bond strength values reach a maximum between 600 and 3500 Further research is needed to more discretely define the optimum molar mass value The availability of oligomers between 1000 M_w and 3000 M_w would permit a refined model for the relationship for this oligomeric series to bond strength To apply this model to general classes of priming oligomers, it will be necessary to use an absolute molar mass determination technique and it might be even more practical to use viscosity or diffusion rate determinations to stratify the priming oligomers This work would then need to be repeated with other families of oligomers to apply these findings to all groups of priming oligomers

The statistical model that was fit to the data might be refined in various ways Lower power ordinal statistical tests were required because the data did not satisfy the equal variance condition If the lowest molar mass and highest molar mass data points are removed, the three remaining data points will generate a straight line For this reason it is desirable in the future to synthesize one or two oligomers with lower molar masses (perhaps target 600 and 400) This would allow a more accurate determination of the relationship between bond strength and molar mass For a linear graph a desirable design criterion is the need to find the smallest monomeric component that provides enough strength to resist fracture If this linear model were confirmed 2-hydroxyethyl methacrylate might be the optimum useful monomer (relative to the material properties) If, on the other hand, a graph that exhibits a maximum molar mass value within the expanded range of experimental treatments then an absolute method of molar mass determination would yield a quantitative molar mass value that could be used as a design criterion for adhesive oligomers

With the selected molar mass values it is unreasonable to assign a certain molecular unit to the difference between the 882 and 998 molar mass groups These molar masses are relative values and represent molar masses of methacrylate run against polystyrene standards The actual absolute quantitivative values represent numbers that are fractions or multiples of the numbers given These values may better represent descriptions of true differences between each group if only one significant figure were used Thus the M_w for the groups would be 900, 1000 3000 5000 and 6000 amu Another explanation for the change in slope of the graph for the lowest two is the potential contamination of one batch by water during the synthesis Exposure to moisture was conscientiously avoided until after the HEMA was added Moisture during the preliminary stages would allow the hydrolysis of the anhydride ring producing either monomeric maleic acid groups, or carboxylate side groups along the oligomer backbone If some of the maleic anhydride rings hydrolyzed the oligomer would have an altered chemistry compared to the other groups With the increase in acidic side groups it would be more hydrophilic and with the decrease in methacrylates it would have a reduced functionality One very useful feature of the oligomer used here is the simplicity of isolating one characteristic of the oligomer (molar mass) while holding all other variables (hydrophilicity acidity chain stiffness functionality, to name a few) constant. This allows the examination of single features without having to consider confounding issues during the analysis.

Future research on this oligomer should focus on discrete examination of different features of a primer in a very controlled fashion. Having established a synthesis protocol, it would be interesting to use other methacrylates to add across the anhydride ring. A first attempt could consist of ethyl-, then butyl- and propyl-hydroxy methacrylate as the side groups added to the ring. The next step would be to examine blends of a methacrylate (to retain double bonds for *in vivo* free radical polymerization with the composite restorative materials) and an amino acid (to enhance wetting of the collagen). Another feature for manipulation is the tert-butyl group that has an ester linkage to the backbone. This tert-butyl group can function as a protecting group for the ester group. If it were hydrolyzed by an SN2 reaction, the backbone would have an increased number of carboxylate side groups compared to the HEMA side group. This reaction would change the hydrophilic/lipophilic nature of the oligomer. This short discussion of the potential of the oligomer could be expanded even more, but just these variations highlight one primary value of the oligomer. It is a potential tool for the determination of many design criteria for bonding agents. As the bond strength values are increased beyond 18 MPa, the altered oligomer would be of interest clinically. By selecting the optimum configuration of the various components of the oligomer, an ideal oligomer can be synthesized to promote adhesion to tooth structure.

With the determination of design parameters, future clinical requirements will be moreeasily satisfied. One issue that is of interest with all biomedical materials is the biocompatibility of the material. If cytotoxicity issues develop with one monomer, design criteria will be used to optimize future polymeric systems, while altering the polymer to decrease cytotoxicity (or allogenicity). The sensitivity of oligomeric alterations on dentin bond strength reported here can also be used in cell culture tests to evaluate the effects of subtle changes in chemistry. This design flexibility also will aid in adapting this oligomer for biomedical acceptability.

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BIOGRAPHICAL SKETCH

Nicole Dean Richards was born Oct. 25, 1945 in Cooperstown, NY. Primary and secondary education was acquired in Auburn, Alabama. She attended Antioch College in Yellow Springs, Ohio from 1964 to 1967. At that time, she left with her husband and returned in 1974 to graduate. For the next five years, she raised her two boys, Ted and David Hempy. In 1979, she returned to school at the University of Kentucky with the intention of attending dental school. In 1983 she graduated from the University of Kentucky College of Dentistry. For the next 7 years she was in private practice part-time and was involved in dental biomaterials research part-time at the University of Kentucky. She was involved with projects on amalgam and posterior composites. In 1990 she became an assistant professor, research track, in the dental college. During the next year and a half, she worked on a clinical and laboratory research project examining the effects of patient age on dentin bonding. In 1991 she became a scientist-in-training at ADA Paffenbarger Research Center at NIST in Gaithersburg, MD. While there, she worked on projects on dentin bonding. In 1992, she went to the University of Michigan for graduate work in dental biomaterials. In 1995 she transferred to the University of Florida to earn a doctorate in Materials Science and Engineering.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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